

CLAIMS

1. A subtilase variant comprising an insertion of at least one amino acid residue between:

- (a) positions 42 and 43;
- (b) positions 51 and 56;
- (c) positions 155 and 161;
- (d) positions 187 and 190;
- (e) positions 216 and 217;
- (f) positions 217 and 218; and/or
- (g) positions 218 and 219;

wherein the positions are numbered according to the amino acid sequence of the mature subtilisin BPN'.

2. The variant of claim 1, wherein the insertion is between positions 51 and 52; positions 52 and 53; positions 53 and 54; positions 54 and 55; or positions 55 and 56.

3. The variant of claim 1, wherein the insertion is between positions 155 and 156; positions 156 and 157; positions 157 and 158; positions 158 and 159; positions 159 and 160; or positions 160 and 161.

4. The variant of claim 1, wherein the insertion is between positions 187 and 188; positions 188 and 189; or positions 189 and 190.

5. The variant of claim 1, where the variant has a residual activity of at least 10% in the Ovo-inhibition assay.

6. The variant of claim 5, where the variant has a residual activity of at least 15%, preferably at least 20%, more preferably at least 25%.

7. The variant of claim 1, wherein the variant contains more than one insertion between said positions.

8. The variant of claim 1, wherein the variant contains two or more insertions between different sets of the above positions.

9. The variant of claim 8, wherein the variant contains more than two insertions between each of said sets of positions.

10. The variant of claim 1, wherein the insertion is selected from the group consisting of X42XA, X42XT, X42XG, X42XS, X42XD, X42XE, X42XK, X42XR, X42XH, X42XV, X42XC, X42XN, X42XQ, X42XF, X42XI, X42XL, X42XM, X42XP, X42XW and X42XY.

11. The variant of claim 2, wherein the insertion is selected from the group consisting of X51XA, X51XT, X51XG, X51XS, X51XD, X51XE, X51XK, X51XR, X51XH, X51XV, X51XC, X51XN, X51XQ, X51XF, X51XI, X51XL, X51XM, X51XP, X51XW and X51XY.

12. The variant of claim 2, wherein the insertion is selected from the group consisting of X52XA, X52XT, X52XG, X52XS, X52XD, X52XE, X52XK, X52XR, X52XH, X52XV, X52XC, X52XN, X52XQ, X52XF, X52XI, X52XL, X52XM, X52XP, X52XW and X52XY.

13. The variant of claim 2, wherein the insertion is selected from the group consisting of X53XA, X53XT, X53XG, X53XS, X53XD, X53XE, X53XK, X53XR, X53XH, X53XV, X53XC, X53XN, X53XQ, X53XF, X53XI, X53XL, X53XM, X53XP, X53XW and X53XY.

14. The variant of claim 2, wherein the insertion is selected from the group consisting of X54XA, X54XT, X54XG, X54XS, X54XD,

X54XE, X54XK, X54XR, X54XH, X54XV, X54XC, X54XN, X54XQ, X54XF, X54XI, X54XL, X54XM, X54XP, X54XW and X54XY.

15. The variant of claim 2, wherein the insertion is selected from the group consisting of X55XA, X55XT, X55XG, X55XS, X55XD, X55XE, X55XK, X55XR, X55XH, X55XV, X55XC, X55XN, X55XQ, X55XF, X55XI, X55XL, X55XM, X55XP, X55XW and X55XY.

16. The variant of claim 3, wherein the insertion is selected from the group consisting of X155XA, X155XT, X155XG, X155XS, X155XD, X155XE, X155XK, X155XR, X155XH, X155XV, X155XC, X155XN, X155XQ, X155XF, X155XI, X155XL, X155XM, X155XP, X155XW and X155XY.

17. The variant of claim 3, wherein the insertion is selected from the group consisting of X156XA, X156XT, X156XG, X156XS, X156XD, X156XE, X156XK, X156XR, X156XH, X156XV, X156XC, X156XN, X156XQ, X156XF, X156XI, X156XL, X156XM, X156XP, X156XW and X156XY.

18. The variant of claim 3, wherein the insertion is selected from the group consisting of X157XA, X157XT, X157XG, X157XS, X157XD, X157XE, X157XK, X157XR, X157XH, X157XV, X157XC, X157XN, X157XQ, X157XF, X157XI, X157XL, X157XM, X157XP, X157XW and X157XY.

19. The variant of claim 3, wherein the insertion is selected from the group consisting of X158XA, X158XT, X158XG, X158XS, X158XD, X158XE, X158XK, X158XR, X158XH, X158XV, X158XC, X158XN, X158XQ, X158XF, X158XI, X158XL, X158XM, X158XP, X158XW and X158XY.

20. The variant of claim 3, wherein the insertion is selected from the group consisting of X159XA, X159XT, X159XG, X159XS, X159XD, X159XE, X159XK, X159XR, X159XH, X159XV, X159XC, X159XN, X159XQ, X159XF, X159XI, X159XL, X159XM, X159XP, X159XW and X159XY.

21. The variant of claim 3, wherein the insertion is selected from the group consisting of X160XA, X160XT, X160XG, X160XS, X160XD, X160XE, X160XK, X160XR, X160XH, X160XV, X160XC, X160XN, X160XQ, X160XF, X160XI, X160XL, X160XM, X160XP, X160XW and X160XY.

22. The variant of claim 4, wherein the insertion is selected from the group consisting of X187XA, X187XT, X187XG, X187XS, X187XD, X187XE, X187XK, X187XR, X187XH, X187XV, X187XC, X187XN, X187XQ, X187XF, X187XI, X187XL, X187XM, X187XP, X187XW and X187XY.

23. The variant of claim 4, wherein the insertion is selected from the group consisting of X188XA, X188XT, X188XG, X188XS, X188XD, X188XE, X188XK, X188XR, X188XH, X188XV, X188XC, X188XN, X188XQ, X188XF, X188XI, X188XL, X188XM, X188XP, X188XW and X188XY.

24. The variant of claim 4, wherein the insertion is selected from the group consisting of X189XA, X189XT, X189XG, X189XS, X189XD, X189XE, X189XK, X189XR, X189XH, X189XV, X189XC, X189XN, X189XQ, X189XF, X189XI, X189XL, X189XM, X189XP, X189XW and X189XY.

25. The variant of claim 1, wherein the insertion is selected from the group consisting of X216XA, X216XT, X216XG, X216XS, X216XD, X216XE, X216XK, X216XR, X216XH, X216XV, X216XC, X216XN,

X216XQ, X216XF, X216XI, X216XL, X216XM, X216XP, X216XW and X216XY.

26. The variant of claim 1, wherein the insertion is selected from the group consisting of X217XA, X217XT, X217XG, X217XS, X217XD, X217XE, X217XK, X217XR, X217XH, X217XV, X217XC, X217XN, X217XQ, X217XF, X217XI, X217XL, X217XM, X217XP, X217XW and X217XY.

27. The variant of claim 1, wherein the insertion is selected from the group consisting of X218XA, X218XT, X218XG, X218XS, X218XD, X218XE, X218XK, X218XR, X218XH, X218XV, X218XC, X218XN, X218XQ, X218XF, X218XI, X218XL, X218XM, X218XP, X218XW and X218XY.

28. The variant of claim 1, wherein the parent subtilase belongs to the sub-group I-S1.

29. The variant of claim 28, wherein the parent subtilase is selected from the group consisting of BSS168, BSSAS, BSAPRJ, BSAPRN, BMSAMP, BASBPN, BSSDY, BLSCAR BLKERA, BLSCA1, BLSCA2, BLSCA3, BSSPRC, and BSSPRD, or functional variants thereof having retained the characteristics of sub-group I-S1.

30. The variant of claim 1, wherein the parent subtilase belongs to the sub-group I-S2.

31. The variant of claim 30, wherein the parent subtilase is selected from the group consisting of BSAPRQ, BLS147 BSAPRM, BAH101, BLSAVI (BLS309), BSKSMK, BAALKP (BAPB92) BLSUBL, BSEYAB, TVTHER, and BSAPRS, or functional variants thereof having retained the characteristics of sub-group I-S2.

32. The variant of claim 31, wherein the parent subtilase is BLSAVI.

33. The variant of claim 1, wherein the variant comprises at least one further modification.

34. The variant of claim 33, wherein the modification is a substitution.

35. The variant of claim 1, wherein the variant further comprises at least one modification in position 27, 36, 56, 76, 87, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 120, 123, 129, 131, 132, 133, 143, 159, 167, 170, 192, 194, 206, 217, 218, 222, 224, 232, 235, 236, 245, 248, 252 or 274.

36. The variant of claim 35, wherein the variant further comprises the modification S101G+S103A+V104I+G159D+A232V+Q236H+Q245R+N248D+N252K.

37. The variant of claim 1, wherein the variant further comprises at least one additional amino acid residue in the active site loop (b) region from position 95 to 103.

38. A cleaning or detergent composition, comprising the variant of claim 1 and a surfactant.

39. The composition of claim 38, which additionally comprises an amylase, cellulase, cutinase, lipase, oxidoreductase, another protease, or combination thereof.

40. A method for removal of egg stains from a hard surface or from laundry, comprising contacting the egg stain-containing hard

surface or the egg stain-containing laundry with a cleaning or detergent composition of claim 38.

41. The method of claim 40, wherein the composition additionally comprises an amylase, cellulase, cutinase, lipase, oxidoreductase, another protease, or combination thereof.

42. An isolated polynucleotide with a DNA sequence encoding a subtilase variant of claim 1.

43. An expression vector comprising the isolated polynucleotide of claim 42.

44. A microbial host cell transformed with the expression vector of claim 43.

45. A microbial host cell of claim 44, which is a bacterium, preferably a *Bacillus*, especially a *B. latus*.

46. A microbial host cell of claim 44, which is a fungus or yeast, preferably a filamentous fungus, especially an *Aspergillus*.

47. A method for producing a subtilase variant, comprising culturing a host of claim 44 under conditions conducive to the expression and secretion of the variant, and recovering the variant.